

Isolation and Morphological Characters of Endophytic Microbes from Some Insulin Plants

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Abstract

In the course of isolation, six endophytic fungi and two endophytic bacteria were isolated from the fresh leaves of some insulin plants belonging the family Cucurbitaceae, Asteraceae, Rubiaceae, Apocynaceae and Lamiaceae, which was carried out by the surface sterilization method (Ando and Inaba, 2004). Plant samples were collected from Hinthada Township, Ayeyarwady Region. Isolated endophytic microbes were designated as AP-1, AP-2, AP-3, AP-4, AP-5, AP-6, AP-7 and AP-8. These isolated endophytic microbes are cultured on agar plate and sub-cultured to obtain pure culture. The morphological characters were studied. Isolated six fungi and two bacteria will be subjected in a preliminary study to find out the identification and antimicrobial activity.

Keywords : Isolation and morphological characters

Introduction

Insulin plant is one of the folk medicines used for the treatment of diabetes. Today people are interested in the use of herbal medicine due to all effects associated with synthetic drugs. Plants have been source of diverse medicinal therapeutic agents (Anonymous, 1967 & Perry, 1980).

Plants may serve as a reservoir of large number of microbes known as endophytes. Endophytes are microbes that inhabit plant hosts for all or part of their life cycle. They colonize the internal plant tissues beneath the epidermal cell layers without causing any apparent harm or symptomatic infection to their host (Selim, 2012). The word endophyte means 'in the plant' (endo Gr. = within, phyton = plant) (Schulz, 2006). All plants in natural ecosystem appear to be symbiotic with fungal endophytes (Rodriguez, 2008).

Approximately, there are near to 30,000 plant species on earth and each individual plant is the host to one or more endophytes, and many of them may colonize certain hosts (Selim, 2012).

The present research paper studies eight species belonging to five families of some insulin plants. Such species were described in detail with their outstanding characters. Plants have been used as antimicrobial agents because of their antimicrobial traits. The microbes that isolated from plant parts are endophytes. Though the meaning of the term "endophytes" varies depending on the researching, it can be defined the endophyte as microbes living inside the healthy plants.

The aim of this research is to study the isolation of endophytic microbes and their morphological characters from the leaves of some insulin plants, the objectives of this research to study the outstanding characters of selected six insulin plants, to screen different isolated microbes from leaves of some insulin plants.

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Materials and Methods

Study site

- Hinthada Township is situated in the Ayeyarwady Region.
- It is bounded on the west by the Bay of Bengal and on the south by the Andaman Sea and the Gulf of Moattama.
- Hinthada Township is situated between North Latitude $17^{\circ} 15'$ and $17^{\circ} 39'$.
- East longitude $95^{\circ} 10'$ and $95^{\circ} 35'$ with an area of 378.695 square miles.

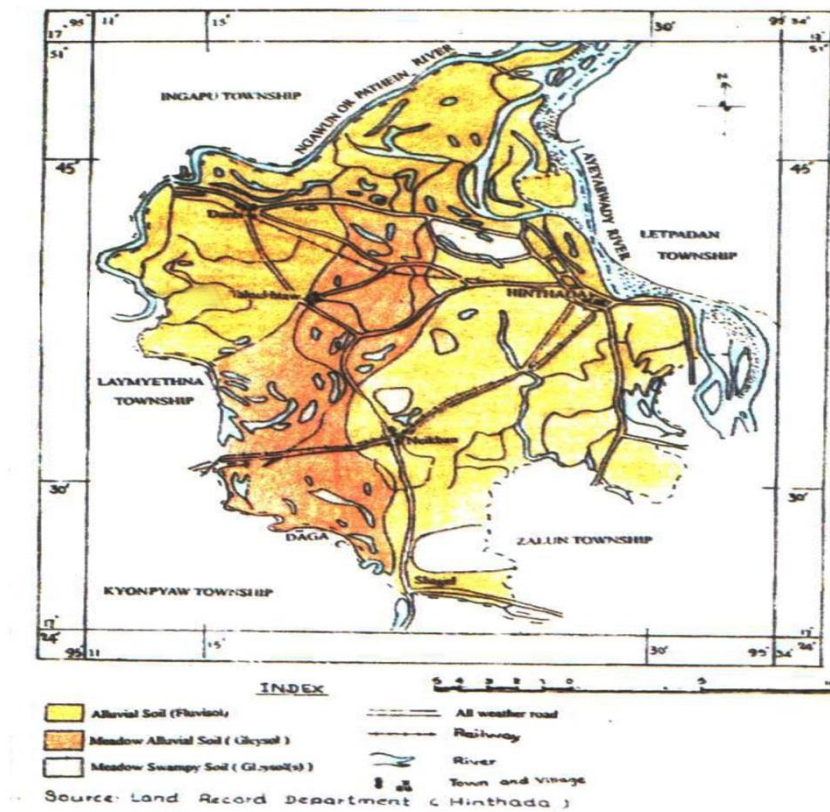


Figure 1. Plant samples collected area (location map of Hinthada Township)

Collection of plant samples

The specimens used in this research were collected from Hinthada Township, Ayeyarwady Region, during the flowering and fruiting period from June to December. The vegetative and reproductive parts of the fresh specimens were identified according to Hooker, (1885), Backer, (1965), and Kress, et al., (2003).

Isolation procedures of endophytic microbes

Surface sterilization method (Ando and Inaha, 2004)

The endophytic microbes are isolated from leaves of some insulin plants the following procedures.

Firstly, the leaves of selected six insulin plants were washed in running tap-water for 15 minutes. Next, the leaves were cut into small pieces of 6×6 mm. Thus, the leaves were sterilized by immersed in 95% ethanol for 15 seconds. The samples were rinsed 3 times in sterile distilled water. Then, the samples were excised into smaller pieces of 5×5 mm and dried

on sterilized paper. These pieces were placed on agar plates and then incubated for 3 to 7 days at room temperature.

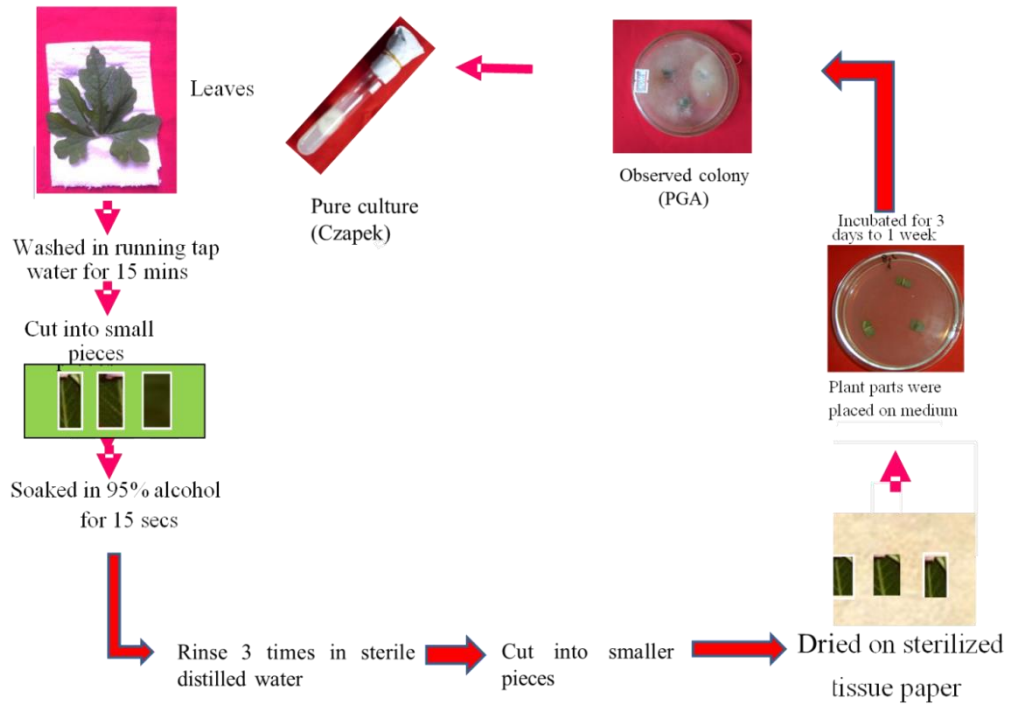


Figure 2. Isolation procedure of endophytes from plant parts (Ando, 2004)

Medium used for isolated endophytic microbes

PGA Medium

(Potato Glucose Agar)

Potato	- 20 g
Peptone	- 0.3 g
Glucose	- 2.0 g
Agar	- 1.8 g
D.W	- 100 mL
pH	- ± 6.5

Czapek Dox Agar Medium

Sucrose	-	3.0 g
NaNO ₃	-	0.2 g
K ₂ HPO ₄	-	0.1 g
MgSO ₄ 7H ₂ O	-	0.05 g
KCL	-	0.05 g
FeSO ₄ 2H ₂ O	-	0.001 g
Agar	-	1.8 g
D.W	-	100 mL
pH	-	± 6.5

After autoclaving, Chloramphenicol (for fungi) and Nystatin (for bacteria).

Results

Table 1. Some insulin plants used for the isolation of Endophytic Microbes

No.	Plant Sources	Myanmar Name	Family
1	<i>Momordica charantia</i> L.	Kyet-hinga	Cucurbitaceae
2	<i>Momordica charantia</i> L.	Aung bala kyet-hinga	Cucurbitaceae
3	<i>Gynura procumbens</i> (Lour.) Merr.	Pyarr-hmee	Asteraceae
4	<i>Morinda citrifolia</i> L.	Ye-yo	Rubiaceae
5	<i>Catharanthus alba</i> (L.) G.Don.	Thinbaw-mahnyo	Apocynaceae
6	<i>Orthosiphon aristatus</i> (Blume)Miq.	Thagya-mageik	Lamiaceae

Outstanding characters of some insulin plants

Scientific Name	-	<i>Momordica charantia</i> L.
Myanmar Name	-	Kyet-hinga
English Name	-	Bitter gourd
Family	-	Cucurbitaceae

Outstanding Characters

Tendrils climbing herbs. Leaves: simple, alternate, petiolate, exstipulate, lamina palmatisect, bases deeply-cordate, margins sinuate-dentate, tip acute. Inflorescences: Axillary and solitary cyme. Flowers: unisexual, monoecious, pedicels long. Male flower: bracteate, pedicellate, ebracteolate, unisexual, regular, actinomorphic, pentamerous, cyclic, epigynous, flowers large and yellow. Female flower: bracteate, ebracteolate, pedicellate, incomplete, unisexual, regular, actinomorphic, pentamerous, cyclic. Fruit: succulent berry pepo, ellipsoid, rostrate, muricate. Seed: Obovate, compressed.



Habit



Leaf



Inflorescence



Male flower



Female flower

Figure 3. Habit, leaf, inflorescence, flower of *Momordica charantia* L.

Scientific Name	-	<i>Momordica charantia</i> L.
Myanmar Name	-	Aung bala kyet – hinga
English Name	-	Bitter gourd
Family	-	Cucurbitaceae

Outstanding Characters

Tendrils climbing herbs. Leaves: simple, alternate, petiolate, exstipulate, lamina palmatisect, base deeply-cordate, margins sinuate-dentate, tip acute. Inflorescences: axillary, 2-3 flowered cymes. Male flower: bracteate, pedicellate, ebracteolate, unisexual, regular, actinomorphic, pentamerous, cyclic, epigynous, flowers small and yellow. Female flower: bracteate, pedicellate, ebracteolate, unisexual, regular, actinomorphic, pentamerous, cyclic, epigynous, flowers large and pale yellow. Fruit: oblong, muriculate. Seed: obovate, compressed.



Figure 4. Habit, leaf, inflorescence, flower of *Momordica charantia* L.

Scientific Name	-	<i>Gynura procumbens</i> (Lour.) Merr.
Myanmar Name	-	Pyarr-hmee
English Name	-	Sambung nyawa
Family	-	Asteraceae

Outstanding Characters

Perennial, succulent herbs. Leaves: simple, alternate, petiolate, exstipulate, lamina elliptical, margins undulate, tip acute. Inflorescences: terminal and axillary corymbose cyme, capitulum, involucre bracts. Ray florets: absent. Disc Florets: bracteate, ebracteolate, sessile, bisexual, actinomorphic, pentamerous, cyclic, epigynous. Fruit: achene black, linear, densely pubescent. Seed: small.

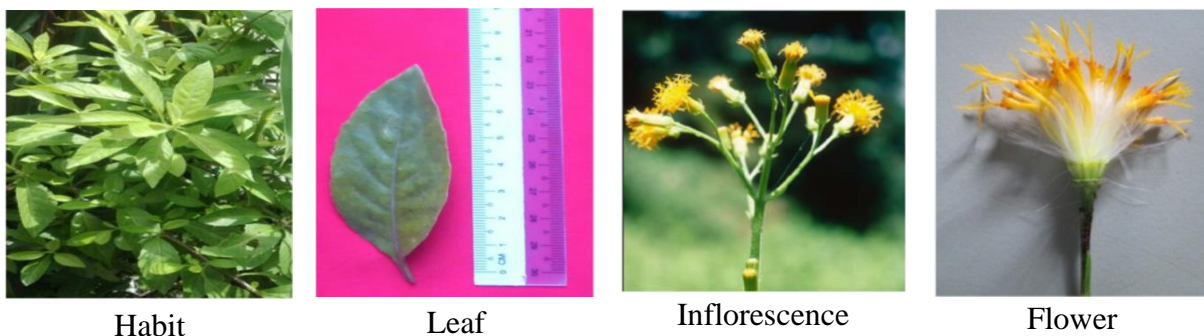


Figure 5. Habit, leaf, inflorescence, flower of *Gynura procumbens* (Lour.) Merr.

Scientific Name	-	<i>Morinda citrifolia</i> L.
Myanmar Name	-	Ye-yo
English Name	-	Noni
Family	-	Rubiaceae

Outstanding Characters

Ever green small trees, latex present. Leaves: simple, opposite and decussate, petiolate, stipulate (interpetiolar), laminae broadly elliptic to obovate, the base acute, margins entire, tip acute. Inflorescences: axillary dense globose head cymes. Flowers: ebracteate, ebracteolate, sessile, complete, bisexual, regular, actinomorphic, pentamerous, white, cyclic, epigynous. Fruit: Aggregate drupe, oblong, irregularly shaped, pale green. Seed: ovoid, brownish.



Habit

Leaf

Inflorescence

Flower

Figure 6. Habit, leaf, inflorescence, flower of *Morinda citrifolia* L.

Scientific Name	-	<i>Catharanthus alba</i> (L.) G. Don.
Myanmar Name	-	Thinbaw – mahnyo
English Name	-	Periwinkle
Family	-	Apocynaceae

Outstanding Characters

Evergreen, herb, latex present. Leaves: opposite and decussate, simple, petiolate, exstipulate, laminae oval to oblong, base obtuse, margin entire, tip rounded. Inflorescences: terminal or axillary cymes. Flowers: bracteate, ebracteolate, sub-sessile, complete, bisexual, regular, actinomorphic, pentamerous, cyclic, hypogynous. Fruit: follicle cylindrical, narrow. Seed: numerous, tiny, blackish – brown.



Habit

Leaf

Inflorescence

Flower

Figure 7. Habit, leaf, inflorescence, flower of *Catharanthus alba* (L.) G. Don.

Scientific Name	-	<i>Orthosiphon aristatus</i> (Blume) Miq.
Myanmar Name	-	Thagya-mageik
English Name	-	Java tea
Family	-	Lamiaceae

Outstanding Characters

Perennial herb, stem erect, quadrangular, pubescent. Leaves: simple, opposite and decussate, petiolate, exstipulate, laminae rhomboid shape, bases cuneate, margins seriate, tips acuminate both surfaces glabrous. Inflorescences: terminal raceme. Flowers: bracteates, ebracteolate, pedicellate, bisexual, zygomorphic, hypogynous. Fruit: nutlet, dark-brown, ovoid. Seed: oblongnoid, dark-brown, glabrous.

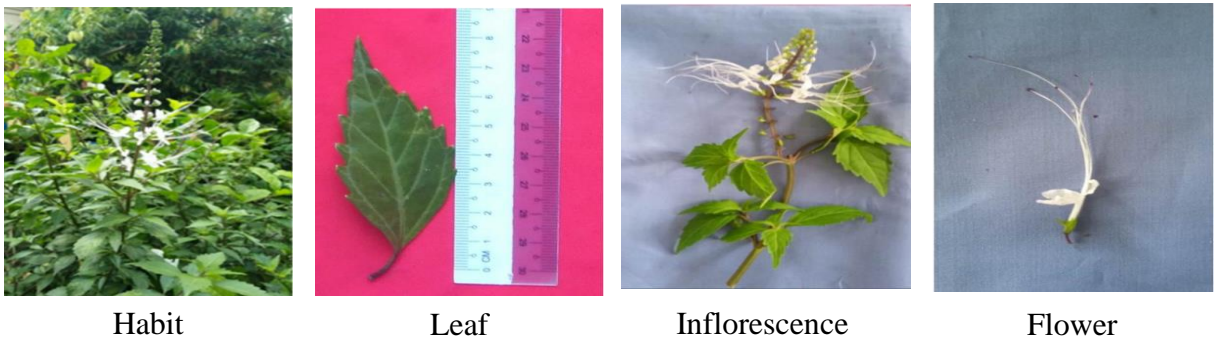


Figure 8. Habit, leaf, inflorescence, flower of *Orthosiphon aristatus* (Blume) Miq.

Isolation of endophytic microbes

The endophytic microbes were isolated from the leaves of some insulin plants by the method of surface sterilization (Ando and Inaba, 2004). The morphological characters were observed by the method of Barnett, 1969, Ando and Inaba, 2004.

Table 2. Endophytic fungi and bacteria isolated from Six kinds of some Insulin plants

No.	Plant Sources	Isolated plant parts	Isolated endophytic microbes	
			Fungi	Bacteria
1.	Kyet-hinga	Leaf	AP-1	-
2.	Aung bala kyet-hinga	Leaf	AP-2	-
3.	Pyarr-hmee	Leaf	AP-3	AP-7, AP-8
4.	Ye-yo	Leaf	AP-4	-
5.	Thinbaw-mahnyo	Leaf	AP-5	-
6.	Thagya-mageik	Leaf	AP-6	-

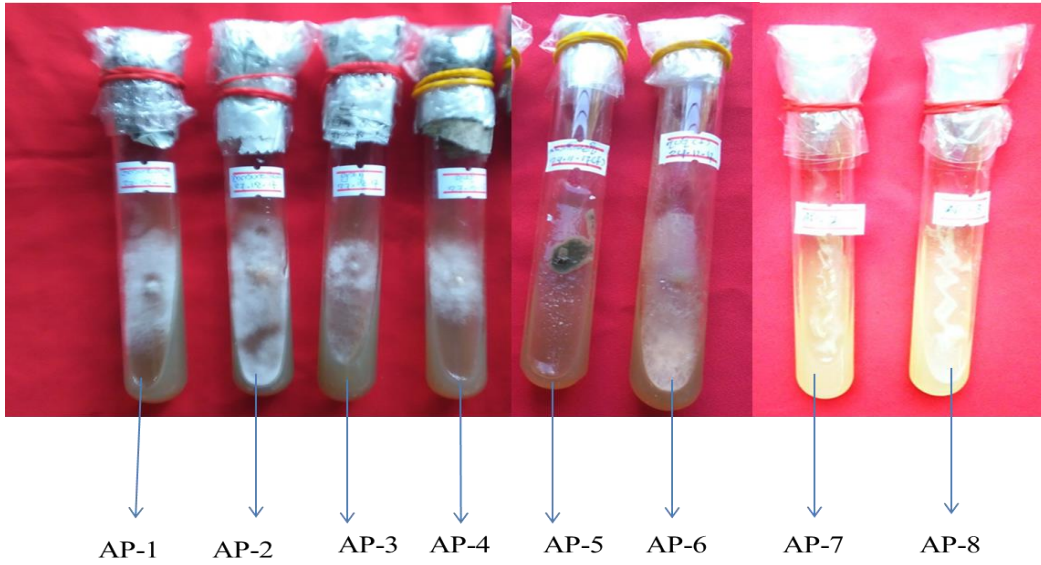
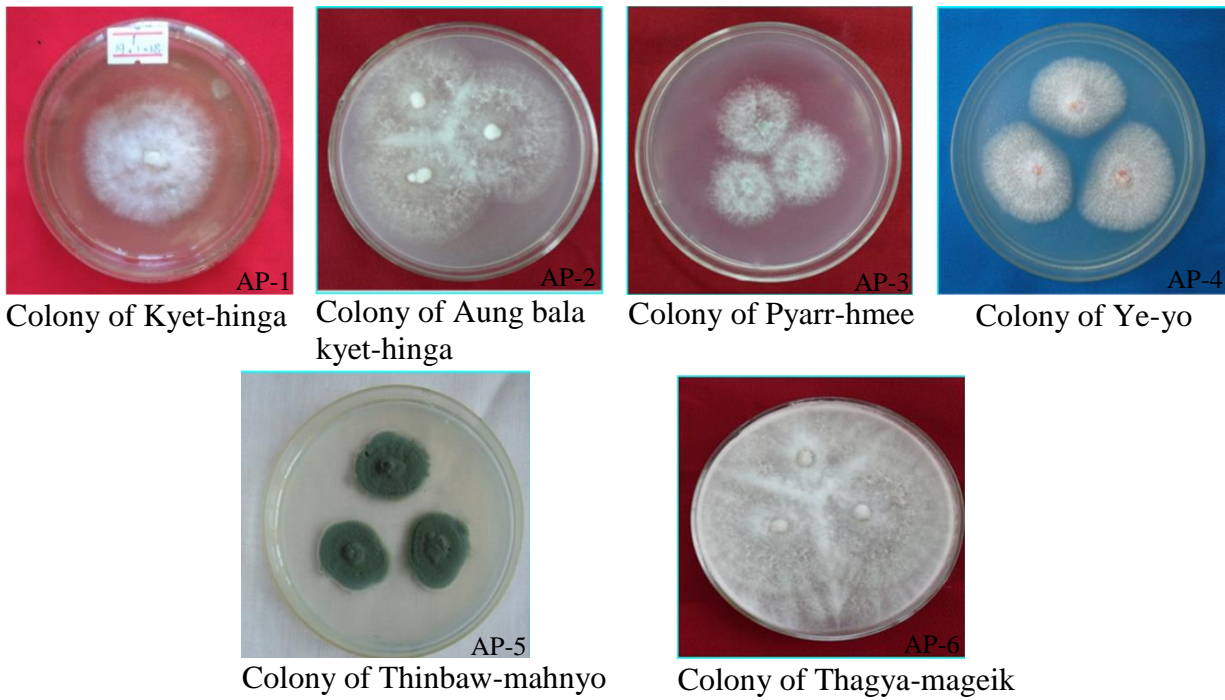


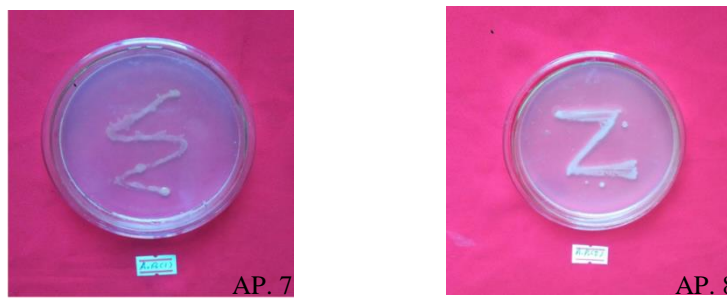
Figure 9. Pure culture of isolated endophytic microbes from some insulin plant leaves
Morphological characters of isolated endophytic six fungi



Colony of Kyet-hinga Colony of Aung bala kyet-hinga Colony of Pyarr-hmee Colony of Ye-yo
Colony of Thinbaw-mahnyo Colony of Thagya-mageik

Figure 10. Morphological characters of isolated fungi (AP. 1 to AP. 6)

Morphological characters of isolated endophytic two bacteria



Cell morphology of Pyarr-hmee Cell morphology of Pyarr-hmee

Figure 11. Morphological characters of isolated bacteria (AP. 7 to AP. 8)

Discussion and Conclusion

Endophytic microbes from some insulin plants were collected from Hinthada Township area, during June to December. In this research, the eight endophytic microbes are isolated from six insulin plants. Among them, six endophytic fungi are isolated from *Momordica charantia* L. (Kyet-hinga), *Gynura procumbens* (Lour.) Merr. (Pyarr-hmee), *Morinda citrifolia* L. (Ye-yo), *Catharanthus alba* (L.) G.Don. (Thinbaw-mahnyo), *Orthosiphon aristatus* (Blume) Miq. (Thagya-mageik) and only two endophytic bacteria are isolated from *Gynura procumbens* (Pyarr-hmee), which was carried out by the method of surface sterilization (Ando and Inaba, 2004).

Eight endophytic microbes of pure culture were selected and preliminarily studied their taxonomical characters and morphology of microbes were described.

Eight endophytic microbes (6 fungi and 2 bacteria) were isolated from 6 kinds of insulin plants. It is concluded that screening of drug resistant strains of human and plant pathogen is essential. Isolated six fungi and two bacteria will be subjected in a preliminary study to find out the identification and antimicrobial activity. They are somehow applied in industrial production of various products such as pharmaceutical for the benefit of Myanmar people.

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